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Assessment of the Genexpert MTB/RIF Assay's Performance for the Quick Identification of Rifampicin-Resistant Mycobacterium Tuberculosis

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Annotation: Introduction: Tuberculosis (TB) remains a global health crisis, and the emergence of rifampicin-resistant TB (RR-TB) has further escalated this challenge. The speed and accuracy of diagnosis are critical for the timely treatment of the disease. Objective: The purpose of this study was to evaluate the diagnostic performance of the GeneXpert MTB/RIF assay in identifying MTB and rifampicin resistance, in an effort to enhance the diagnosis and treatment of tuberculosis (TB).Materials and Methods: This cross-sectional study was conducted at the National Tuberculosis Institute (NTI)/National Reference Laboratory (NRL) for Tuberculosis in Baghdad from February to September 2021. Morning sputum samples were collected from consenting patients. These samples were analyzed using Ziehl-Neelsen staining microscopy, the Xpert MTB/RIF assay, and culture on Löwenstein-Jensen (LJ) medium. Results: Among 140 suspected TB patients, 60 were confirmed to have active pulmonary tuberculosis (ATB) based on at least one positive microbiological test result, including acid-fast bacilli (AFB) staining, bacterial culture, molecular testing (Xpert MTB/RIF), clinical symptoms, and chest radiography (CXR) indicative of TB. Out of the 140 sputum specimens, 38 (27.14%) were positive by ZN/AFB smear microscopy, 60 (42.86%) by the GeneXpert MTB/RIF assay, and 41 (29.28%) by MTB culture on LJ medium. With respect to the detection of bacillary load, out of the sixty specimens that tested positive for bacillary load, the semi-quantitative results showed that 11 samples (18.3%) had high results (mean cycle threshold (CT) value =14.3), 25 (41.7%) had medium results (mean CT value =20.8), 15 (25.0%) had low results (mean CT value = 25.4), and 9 (15.0%) had very low results (mean CT value =30.1).Rifampicin resistance was detected in 7 out of 60 (11.67%) of the MTB-positive specimens, while 53 (88.33%) showed no rifampicin resistance (Rif-S).

Conclusion: The GeneXpert MTB/RIF assay demonstrates high sensitivity and specificity, making it a valuable diagnostic tool for the rapid detection of TB bacilli and the simultaneous identification of RR-TB. Early detection of rifampicin resistance is crucial in curbing the spread of multidrug-resistant TB (MDR-TB) strains.

Keywords: GeneXpert MTB/RIF assay, Mycobacterium Tuberculosis, bacillary load, rifampicin resistance TB (RR-TB).

Introduction

Tuberculosis (TB) is a worldwide affliction and still predominates in high-risk countries like Rifampicin-resistant TB (RR-TB). Prompt and accurate diagnosis is key to the treatment as well control of disease. The advent of the World Health Organization (WHO) approved GeneXpert MTB/RIF assay has changed the faces of TB diagnostics with a rapid, sensitive and specific test for Mycobacterium tuberculosis (MTB), as well rifampicin resistance which serves as a proxy indicator to replacements MDR-TB that is multidrug-resistant TB (WHO, 2013). GeneXpert MTB/RIF is a molecular real-time polymerase chain reaction (PCR) test that detects Mycobacterium tuberculosis DNA and the most common mutations in rpoB gene causing resistance to rifampin. The test on sputum samples is faster than reference culture methods and can produce results only 2 hours (Boehme et al., 2010). Cycle threshold (Ct) values are used to measure the MTB bacillary load in GeneXpert MTB/RIF assay platforms. During an analysis of pairing on lymph node specimens this Ct value was

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inversely related to bacillary load; the lower (earlier) CT value is indicative of higher bacterial burden, and vice-versa (Lawn et al., 2011). This provides an important quantitative assessment of disease severity (Horne, 2010) which is invaluable in monitoring treatment response and making prognostic predictions for the patient. Mutations in the 81-bp "core" region of rpoB, coding for beta subunit of RNA polymerase enzyme detect rifampicin (RIF) resistance. Mutations in this region prevents binding of rifampicin to the RNA polymerase and hence it fails to have its action. One of the new tests for GeneXpert MTB/RIF is that it can detect these mutations with a high sensitivity and specificity (Helb et al.,2010). The importance to clinical practice of this rapid identification not only that an infection is due technically MTB but also because an isolates will be resistant rifampicin, has been demonstrated in the GeneXpert MTB/RIF assay. Prompt detection of RR-TB is essential for the initiation of appropriate treatment regimens, which may improve patient outcomes and reduce transmission to others (Steingart et al., 2014). In addition, estimating bacillary load enables clinicians to make more informed decisions on treatment plans and follow up of the efficacy for therapy (Dorman et al., 2012). The utilized technique to probe the bacillary load and rifampicin resistance in tuberculosis (RR-TB) patients by means of GeneXpert MTB/RIF semi quantitative assay on the basis this study was evaluated aim. The study aims to evaluate the diagnostic accuracy of this molecular test in detecting Mycobacterium tuberculosis and taking Resistance, thus contributing towards better management with a rational diagnosis for Tuberculosis, especially within high-burden regions.

Materials and Methods

Study design:

An investigation that was cross-sectional in nature was carried out at the National Reference Laboratory for Tuberculosis in Baghdad between February and September 2021.

Study population:

Inclusion criteria:

Patients presenting with symptoms suggestive of pulmonary TB defined as those who had any of the following signs or symptoms in the last one month: Chronic cough, hemoptysis, weight loss, or night swears, aged 18 or older, and provided informed consent.

Exclusion criteria

Patients had already initiated anti-TB therapy, Extra pulmonary TB, or other respiratory infections not suspected to be tuberculosis.

Digesting sputum samples in vitro

Host microbiota contaminates sputum samples. Contaminated samples require intensive decontamination to liquefy a greater fraction of organic material and eliminate the bulk unwanted domestic flora. The 4% NaOH (Modified Petroff) method was employed for digestion and decontamination. We have written that it is well known to be equally toxic for contaminants and tubercle bacilli, so you need to keep strictly about the indicated timings. Mycobacterium tuberculosis was grown following reference (David et al., 2018).

Automated molecular detection of Mycobacterium tuberculosis by GeneXpert MTB/RIF Assay

A fully automated cartridge-based molecular system, similar to Cepheid GeneXpert, it is small and entirely automated for processing samples and amplifying nucleic acid sequences for subsequent discovery. The MTBC rpoB gene's wild type, rifampin-susceptible sequence is the target of nucleic acid probes used in this assay to determine whether the gene is present or absent. To cover every unique nucleic acid sequence found in the amplified rpoB gene, five drafted beacons are specifically employed (Fouda et al., 2019). Cepheid's Xpert MTB/RIF Assay, designed for use with their GeneXpert system, is an in-vitro diagnostic test.

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Ethical approval

Verbal agreement was granted by every individual recruited prior to sample collection for this study. This study was passed on a publication ethics committee at the University of Babylon and it was approved.

Statistical analysis

The statistical analysis of the data was conducted using SPSS version 25 by applying chi-square tests, frequency distributions and r coefficient of correlation in this study.

Results

Among 140 suspected patients with TB, 60 cases were identified as active pulmonary tuberculosis (ATB) using a minimum of one microbiologically supported positive, acid-fast bacilli staining results, positive bacterial culture or Xpert MTB/RIF and the presence clinical symptoms and signs, radiography findings that are suggestive implicate tubercle bacillus infection according to CXR (Kaewseekhao et al., 2020). In the present study, of all 140 sputum specimens tested laboratories results positive rates were (27.14%), (42.86%) and (29.28%) by acid fast bacilli smear microscopy, Gene Xpert MTB/RIF assay and mycobacterium tuberculosis culture on LJ medium , respectively as illustrated in table No1.

Table (1): Laboratory diagnostic Techniques Results

Results	Type of methods		
	AFB smear microscopy	Gene Xpert MTB/RIF	Culture
Positive, n (%)	38 (27.14)	60 (42.86%)	41 (29.28%)
Negative, n (%)	102 (72.86)	80 (57.14%)	99 (70.72%)
Total, n (%)	140 (100%)	140 (100%)	140 (100%)

n: number of cases

Detection of bacillary load and rifampicin resistance TB (RR-TB) by GeneXpert MTB/RIF assay

When the test detects MTB, Semi-quantitative results from Xpert are displayed as very low, low, and medium high. GeneXpert software version 4.8 was used for the results interpretation. based on the measured fluorescence signals and computation algorithms embedded, which are presented to the user through a form called view results displayed as table(2), (3).

Table (2 (: MTB DETECTED and RIF Resistance NOT DETECTED results by GeneXpert MTB/RIF assay

Test Result: MTB DETECTED HIGH; Rif Resistance NOT DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	14.3	205	POS	PASS
Probe C	13.3	249	POS	PASS
Probe E	14.6	119	POS	PASS
Probe B	13.9	129	POS	PASS
SPC	25.6	252	NA	PASS
Probe A	12.6	150	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

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Table)3 (: MTB DETECTED and RIF Resistance DETECTED result by GeneXpert MTB/RIF assay

Test Result: MTB DETECTED MEDIUM; Rif Resistance DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	22.7	202	POS	PASS
Probe C	21.7	224	POS	PASS
Probe E	0.0	-7	NEG	PASS
Probe B	22.9	110	POS	PASS
SPC	23.9	295	NA	PASS
Probe A	21.4	127	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

According to the results presented in Tables (4) and (5), among the 60 samples in this study that tested positive for the GeneXpert MTB/RIF test, the semi-quantitative results showed that 11 samples (18.3%) had high results (mean cycle threshold (CT) value =14.3), 25 (41.7%) had medium results (mean CT value =20.8), 15 (25.0%) had low results (mean CT value 25.4), and 9 (15.0%) had very low results (mean CT value =30.1).

Table (4): Frequency of MTB and Rifampicin Resistance detected by GeneXpert MTB/RIF assay in patients with Active Pulmonary TB.

MTB DETECTED by GeneXpert MTB/RIF assay			
	MTB detected	No.	%
1.	high, *n* (%)	11	18.3
2.	low, *n* (%)	15	25.0
3.	Medium, *n* (%)	25	41.7
4.	very low, *n* (%)	9	15.0
5.	total, *n* (%)	60	100
Rif. Resistance detected by GeneXpert Dx. System			
1.	Rif.R, , *n* (%)	7	11.67
2.	Rif.S, *n* (%)	53	88.33
3.	Total	60	100.0

Table (5): Average cycle threshold (CT) value in GeneXpert MTB/RIF assay

N=60	Mean	Std. Deviation	Minimum	Maximum
Low	25.40	1.96	22.10	29.90
Medium	20.82	1.73	17.30	23.20
Very low	30.11	2.71	22.50	34.30
High	14.36	1.79	9.20	17.20

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Discussion

The rapid detection of MTB has been overdue, and a high sensitivity/specificity is required in early diagnosis. The sensitivity of smear microscopy may be lower due to a higher detection threshold because 5000–10,000 bacilli per mL is necessary in specimens to show available positive results when compared with that the GeneXpert MTB/RIF assay needs (Habte et al.,2016). In addition this test needs 3-day early morning sputum for increased sensitivity. On the other hand sputum smear microscopy has low sensitivity as well it cannot differentiate between MTB and MTB complex (Riaz *et al*.,2016).

ZN staining is the traditional method of detection because it is quick, easy to use at low cost and has a high specificity for identifying Mycobacterium tuberculosis infection (Caulfield and Wengenack, 2016). In this study AFB smear microscopy could correctly diagnose 22/140 (15.71%) cases missed by sputum GeneXpert MTB/RIF test. A false-negative diagnosis of smear-negative pulmonary TB may impose considerable financial burden on individuals and their families as well as the society. Conversely, GenexpertMTB/RIF assay showed high sensitivity and specificity for the detection of smear negative pulmonary tuberculosis (Rasheed *et al.*, 2019).

The gold standard method for TB detection is a culture technique that uses Lowenstein Jensen (LJ) medium for mycobacterial growth. The main disadvantage of tuberculosis culture is its long culture cycle, which takes longer than expected and typically takes 3-5 weeks with high sensitivity. This means that it cannot meet the needs of clinical diagnosis. (Sambarey *et al*., 2017). When 10 viable bacilli per milliliter of sputum were detected, the effectiveness of MTB culture on LJ medium was shown (Munir et al.,2015). The reasons for the negative results in culture can be explained by the fact that AFB culture can be identified at lower bacillary loads and can also be influenced by other circumstances, such as specimen collection and processing. According to Shi *et al*. (2018), the GeneXpert MTB/RIF assay is not susceptible to cross-contamination, whereas the MTB culture assay is. This may be another reason contributing to the reduced recovery rate via culture.

A positive Xpert MTB/RIF reading requires a minimum of 131 mycobacterial colony-forming units to be present in the specimen, which makes the GeneXpert assay much more sensitive than ZN smear microscopy for the detection of MTB in pulmonary samples (Atehortúa *et al*., 2015).

For all suspected instances of pulmonary tuberculosis or newly diagnosed patients, the World Health Organization advises sputum GeneXpert MTB/RIF testing (Gilpin *et al*., 2018). The Gene-Xpert MTB/RIF assay may identify M. tuberculosis and the existence of rifampicin resistance directly and concurrently from clinical specimens in less than two hours, as opposed to culture. Although commonly employed in the diagnosis of tuberculosis (TB), nucleic acid amplification tests (NAATs) are unable to distinguish between living and dead bacilli. Although it takes time, live bacilli can be isolated using culture techniques (Wang *et al*., 2020).

In conclusion, the GeneXpert assay is regarded as a useful diagnostic tool for the quick identification of MTB and the concurrent identification of RR-TB. Since early detection makes it easier to stop the spread of the illness and initiate TB treatment on time, the GeneXpert assay's launch has significantly decreased the number of MDR-TB patients.

Mycobacterium tuberculosis (MTB) can be detected using the automated Real-Time polymerase chain reaction assay GeneXpert MTB/RIF, which measures the threshold-cycle (Ct) of many probes that target the rpoB gene to estimate mycobacterial load (Boakye-Appiah *et al*., 2016). The 81 base pair "core" region of the rpoB gene is amplified by the primers used in the Xpert MTB/RIF assay. The entire 81-bp core is examined by five distinct colored fluorogenic nucleic acid hybridization probes, also referred to as molecular beacons (El-Hajj *et al*., 2001). Every molecular beacon was created to be so precise that, even in cases when the target sequence deviates from the wild-type rpoB sequence by just one nucleotide, it will still not bind to its intended target. Molecular beacons only emit light when they are attached to their targets, which is the wild-type rpoB sequence. Therefore, the assay

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distinguishes between the conserved wild-type sequence and mutations in the core region linked to RIF resistance when any one of the five probes is absent (Alame-Emane *et al*., 2017).

Ct values were noted for every one of the five probes. Xpert quantified the bacilli using the five probes (A, B, C, D, and E); the result was given as the Mean Ct value (Najjingo *et al*., 2019).These outcomes agreed with those of other published research. The semiquantitative mycobacterial load results, according to a study by Fradejas et al. (2018), were as follows: Geleta et al. (2015) reported a variety of semi-quantitative mycobacterial load outcomes, such as high (Ct < 16), medium (Ct 16–22), low (Ct 22–28), and extremely low (Ct > 28). The results were also semi-quantitatively low (average CT value of 27.0), semi-quantitatively low (average CT value of 33.1), and medium (average cycle threshold (CT) value of 14.5). Semi-quantitation of bacillary load by Xpert test is based on threshold-cycle (Ct); low Ct value is 22–28; very low Ct value is >28. This is demonstrated by Singh *et al.* (2016).

There is an inverse relationship (r = -0.94) between mycobacterial load and GX Ct. According to Bodmer and Ströhle (2012), higher Ct values indicate a lower concentration of DNA template, whereas lower Ct values indicate a higher initial concentration of DNA template. Bacterial load measurements have long been crucial to the diagnosis of tuberculosis. Clinically helpful for evaluating the risk of transmission and establishing the severity of the disease are semiquantitative measurements of the quantity of Mycobacterium TB bacilli present in a clinical sample (Blakemore *et al*., 2011).

Of the 60 cases with MTB that GeneXpert identified, 11.67% (7/60) of the specimens had rifampicin resistance. Table (4) displays the RIF resistance not detected (Rif.S) values obtained from 88.33% (53/60) of the specimens. The current study's incidence of rifampicin resistance was in line with a study by Aljanaby et al. (2022) that was carried out in the Baghdad governorate and showed that 11.1% of patients with active tuberculosis had RR-TB. This incidence, however, is significantly different from the prevalence rate (14.3%) in Baghdad reported by Al-Obaidy *et al*. (2013). Al-Mussawi *et al*.(2017) reported the frequency of rifampicin upto 7.56% in Basra province south of Iraq . More research is needed in Iraq to determine if this gap is real or an artifact of the methodology involved.

Rifampicin (RIF) belongs to bactericidal drugs and is essential for the treatment of tuberculosis. Up to 95% of RIF-resistant mutations occur in the rpoB gene, encoding for one subunit of RNA polymerase and the site attacked by RIF. The most contributions of rpoB gene mutants are located in an 81 kb rifamycin resistance determining region (RRDR) (Zaw et al.,2018).

Rifamycins detection is a surrogate marker for identifying multiresistant Mycobacterium tuberculosisto rifampicin and to at least another first-line antituberculosis drug (Dagnra et al.,2015). The biggest impetus to MDR-TB epidemics is the delay in recognition of drug resistance leading to delayed treatment and ineffective therapy. The rifampin resistance-determining region (RRDR) of the rpoB gene contains missense mutations that associate with how strains develop Rifampicin resistant mechanisms(Masenga et al.,2017).

Although mutations in rpoB mediating RIF resistance are less frequent than such for other anti-TB agents, the increasing utilization of this agent is helping to drive its rising drug-resistance rate. There is an increased risk of a history of failure to complete treatment regimens lasting longer than one month becoming acquired rifampicin resistance (Mulisa et al., 2015). Dominant growth of known drug-resistant mutants in an 'in vitro history' is plausible because prior ineffective anti-TB regimens only impede the spread of susceptible bacilli, with negligible impact on other resistant strains(Mekonnen et al.,2015) . RR-TB infections will eventually give rise to MDR-TB. The correlation between contact with a known TB patient and MDR-TB was also significant, as evidenced by several other studies that supported the notion that exposure to resistant TB strains causes contact with a known TB patient to result in rifampicin resistance (Desissa et al., 2018). The early detection of rifampicin resistance may reduce the frequency of multidrug resistant (MDR) strains in tuberculosis patients.

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Conclusion: Rapid identification of bacillary load and rifampicin resistance has been made possible by the GeneXpert MTB/RIF assay, which has completely changed the landscape of tuberculosis diagnostics. Its rapid turnaround times and high sensitivity and specificity make it a crucial tool in the fight against tuberculosis (TB), especially in areas with low resources where the illness is most prevalent.

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